

## **Exhibit 6**

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OBSERVATORY

## Pumping Up, the Land Crab Way

By HENRY FOUNTAIN

To shed its shell for a new one, a crab needs a swelled head. And a swelled torso, legs and claws, for that matter. Expanding the body makes the old exoskeleton crack, so the crab can worm its way out of it. But then the crab needs to keep expanding so the new soft shell it secretes is bigger than the old one. Molting is all about growth, after all.

Aquatic crabs expand by taking in more water, creating a temporary hydrostatic "skeleton" of pressurized water that supports the body while the new shell hardens. But what about crabs that spend their time on land?

Jennifer R. A. Taylor, a doctoral student at the University of North Carolina, and her adviser, William M. Kier, have discovered that one land crab, at least, uses air. But it doesn't just pump itself up like a ball; it combines air pressure with water pressure.

"It's kind of like blowing up a balloon inside the body," Ms. Taylor said.

She and Dr. Kier studied red land crabs, *Gecarcinus lateralis*, which are found in the Caribbean and other tropical regions. They measured the pressure within the crab at various points in the molting process. Their findings were published in *Nature*.

These crabs, whose bodies are about three inches wide, can take up only small amounts of water when they are on moist sediments. So to molt, the crab takes in air, trapping it in a cavity right behind the head. This

inflated gut then puts pressure on the hemolymph, the bloodlike fluid within the crab.

Because crabs have an open circulatory system, pressurizing the hemolymph causes expansion throughout the body and provides the stiffness and support the crab needs while the shell hardens.

How do the crabs take in the air? The researchers suspect they swallow it, which is what many molting insects do.

While this pneumo-hydrostatic skeleton (as the researchers call it) provides support, it makes the crab something less than a spring chicken, lacking its normal agility. "The crab's body is designed to work as a rigid system," Ms. Taylor said. During molting, she added, "it is less efficient because that same design is being used as a hydrostatic system."

The lack of agility may also be a behavioral response, she said. The more the crab moves, the greater the risk that some deformation will become permanent as the shell hardens.

"Lots of crabs live on land to some extent," Ms. Taylor said, adding that as these crustaceans became more terrestrial, "using air rather than water would be more important."

### **Keeping the DNA Clean**

With potential bioterrorism agents like the bacterial toxins that cause cholera and botulism, a little can go a long way. That raises problems for biologists devising ways to detect them. How do you find tiny amounts of toxin in food, say, or water?

One approach is to link the toxin to fragments of DNA and then, using the lab technique called PCR, or polymerase chain reaction, amplify the fragments until there are enough to easily detect. But PCR requires that samples be kept extremely clean; contamination by other DNA makes the test less sensitive.

Jeffrey T. Mason of the Armed Forces Institute of Pathology and colleagues have now devised a PCR test for cholera and botulism that reduces the contamination problem by encapsulating the DNA in liposomes, hollow droplets of fat. Only after the sample has been repeatedly cleaned is the DNA released and amplification takes place. The result is a rapid and sensitive field test that is effective with samples containing as few as 10 toxin molecules.

Here's how the process works, as described in a paper in *Nature Biotechnology*. Monoclonal antibodies tailored for a particular toxin are put in the well of a testing plate, where they accumulate on the surfaces. A solution containing the toxin is added, and the toxin molecules bind with the antibodies.

Then the liposomes are added. Each liposome's hollow center contains about 60 copies of a DNA fragment, while the exterior is coated in another toxin-linking molecule. This binds the liposome to the toxin, and the liposome-toxin-antibody combinations stay put while everything else is washed away.

Adding a simple detergent breaks the liposomes open, releasing the DNA, which is then replicated in a process called real-time PCR, producing a fluorescent signal that can be detected. The whole process takes about three hours.

### **The Right Mix for Cells**

Culturing cells in the lab — tumor cells, perhaps, or stem cells — is normally done on a flat surface like glass. But the real world exists in three dimensions, not two. Because cells respond to cues from surrounding cells, it makes sense that the response may be more natural, and the resulting tissue may better mimic the real world, if cells are surrounded in three dimensions rather than two.

But building a three-dimensional matrix for culturing tissue is not easy. A new technique has been developed by Sageeta N. Bhatia and Dirk R. Albrecht of the Massachusetts Institute of Technology and colleagues.

As described in the journal *Nature Methods*, the technique uses a gel that sets by ultraviolet light.

If you are making a chocolate chip cake, the chips will all settle to the bottom unless you distribute them by stirring so they can be locked in place when the cake bakes. Similarly, in their 3-D culturing technique, the researchers had to come up with a way to distribute the cells — cartilage cells from cows — in the liquid gel before it sets.

The method they came up with was to put the cells in a layer of the gel solution sandwiched between two electrodes. The electrodes create nonuniform electric fields in various patterns that can propel the cells sideways and upward, even though they are not electrically charged.

Once the cells are precisely distributed, they are locked in place by formation of the gel. The gel allows nutrient transport, so the technique may prove to be a good way to study the growth of certain tissues under more lifelike conditions.

### **An Encouraging Sight**

A group of North Pacific right whales has been spotted in the Bering Sea. That's good news for those concerned about this species, perhaps the most endangered whale species in the world.

The whales were nearly hunted to extinction in the 1800's and again in the 1960's. Since then, at most six had been seen in any one year.

But in August and September 2004 sonobuoys picked up calls from whales, which were photographed and in one case, tagged with a radio device. In all 17 whales were spotted, including 7 females and 2 calves. The sightings offer hope that the species may be able to recover.